GLUCOSAMINE AND METHOD OF MAKING GLUCOSAMINE FROM MICROBIAL BIOMASS

Cross Reference to Related Applications

The present application is a continuation-in-part of copending U.S. Patent Application No. 10/326,549 filed December 19, 2002, which is a continuation of U.S. Patent Application No. 09/785,695 filed February 16, 2001, and claims priority from PCT Application No. PCT/US02/04468 filed February 15, 2002, each of which is incorporated herein by reference.

Field

The present invention is directed to glucosamine compositions and to methods of making glucosamine compositions.

Background

Glucosamine is a nutraceutical supplement that has been shown to provide significant therapeutic relief for arthritis and joint pain. Although the mechanism is not entirely known, it is believed that glucosamine functions to aid in restoration of the cartilage to relieve inflammation in the joints, thereby providing significant benefit to patients.

Presently, glucosamine is primarily derived from harvested natural sources, such as shellfish and other aquatic organisms. Components of the shell or exoskeleton of these organisms are converted into glucosamine using various production techniques. These natural sources are acceptable for producing glucosamine for some applications, but they have limitations. These limitations include the fact that wild shellfish can have significant variations in their composition because they grow naturally under uncontrolled circumstances. The shellfish can vary in such aspects as their size and composition depending upon the growing conditions as well as their species. Also, without control over the growing conditions, the shellfish can be exposed to environmental contaminants, including heavy metals, that can be retained in glucosamine or other products produced from the shellfish. Shellfish harvests are often seasonal, and thus the supply and price of shellfish shows significant variation over time.

A further concern with glucosamine derived from shellfish is that significant portions of the human population have shellfish allergies and are unable to use products that contain ingredients derived from shellfish. A large percentage of shellfish allergens are specific proteins. Shellfish allergens, such as muscle proteins (e.g., tropomyosin) are found in glucosamine derived from the shellfish sources. It is not economically practical, if even possible to ensure that glucosamine products derived from shellfish sources are completely free of all traces of shellfish allergens. Thus, hyper allergenic individuals who must avoid all shellfish products cannot ingest materials derived from shellfish, such as glucosamine.

An additional problem associated with existing sources of shellfish-derived glucosamine is that some of the shellfish supply is harvested from the seas and oceans of the world. Excessive harvest of shellfish could have a great negative environmental impact. Thus, it is believed that some consumers would prefer to use glucosamine that is not harvested at the expense of sea life. Even if the environmental impact of harvesting shellfish is not negative, there remains concern that the supply of wild shellfish is limited in quantity and inconsistent in quantity from year to year.

Another problem associated with glucosamine compositions derived from shellfish is that such compositions are not "kosher." "Kosher" means fit or proper, and is generally used to describe foods that are prepared in accordance with special Jewish dietary laws. Many people that practice Judaism will only ingest kosher products. All shellfish are non-kosher foods and thus all products derived from shellfish are not considered kosher. Although for certain medicinal applications, a shellfish glucosamine product can receive special dispensation such that it is considered kosher, specially dispensed kosher shellfish-derived glucosamine may be used for medicinal applications only and even then may only be ingested in pill or tablet form. Accordingly, a "fully certified kosher" glucosamine composition (i.e., a kosher product not requiring special dispensation or restricted to medicinal uses in pill or tablet form) is needed. Likewise, many vegans require an animal-product free glucosamine composition and glucosamine compositions derived from shellfish do not meet their dietary needs.

Therefore, a need exists for a source of safe, kosher, non-animal product derived, high-quality glucosamine compositions that can be created economically and with a minimum of environmental impact.

Summary

Disclosed are glucosamine compositions, including glucosamine composition products suitable for human or animal consumption. The disclosed glucosamine compositions are derived from fungal biomass containing chitin. Suitable starting materials include microbial fungal sources, such as fungal sources derived from *Aspergillus sp., Penicillium sp., Mucor sp.,* and combinations thereof. Use of a fungal biomass results in high quality glucosamine compositions that are generally uniform with low levels of impurities. The glucosamine compositions normally have relatively low ash content, and are free of or substantially free of heavy metal contaminants. In addition, as a product of fungal biomass, the glucosamine compositions do not pose a hazard to persons who have shellfish allergies. That is, tropomyosin and other such muscle-derived proteins are not present in fungal biomass. Because the disclosed glucosamine compositions are not derived from shellfish (or any animal source), the disclosed compositions are both kosher and may be consumed by strict vegetarians. Shellfish and products derived from shellfish are not considered kosher by any guidelines regarding kosher products.

Particular embodiments of the disclosed glucosamine compositions comprise glucosamine and no shellfish allergens. Other embodiments of the disclosed glucosamine compositions include kosher glucosamine. Other embodiments of the disclosed glucosamine compositions comprise glucosamine and an absence of animal-derived products. Yet other embodiments of the disclosed glucosamine compositions comprise glucosamine and melanoidins. Further embodiments of the disclosed glucosamine compositions comprise glucosamine, melanoidins, and/or levulinic acid. Other embodiments of the disclosed glucosamine compositions have lipophilic oxygen radical absorbance capacity (ORAC) values of from 30 μmole TE/g to 150 μmole TE/g or from 35 μmole TE/g to 100 μmole TE/g or from 35 μmole TE/g to 50 μmole TE/g.

Also disclosed are various methods for producing glucosamine compositions by acid hydrolysis of fungal biomass. The methods for obtaining glucosamine compositions from microbial biomass include, for example, reacting chitin-containing biomass in a relatively concentrated acidic solution at a relatively elevated temperature. Also disclosed are methods for obtaining glucosamine compositions from microbial biomass by, for example, reacting the chitin-containing biomass in a relatively mild acidic solution and then in a relatively concentrated acidic solution. In an alternative embodiment, the microbial chitin-containing biomass is reacted with a basic solution before or after acid hydrolysis treatment. In yet another embodiment, fungal biomass is treated with an acidic solution at an elevated temperature and/or pressure to produce glucosamine compositions.

Drawings

FIG. 1 is a prior art flow diagram illustrating a process for producing glucosamine from shellfish.

FIG. 2 is a flow diagram of one of the disclosed methods for producing particular embodiments of the glucosamine compositions.

FIG. 3 is a flow diagram of another of the disclosed methods for producing embodiments of the glucosamine compositions.

FIG. 4 is chart showing the percent yield of glucosamine in an embodiment of the disclosed glucosamine composition produced using an embodiment of the glucosamine composition methods.

FIG. 5 is a chromatogram of an embodiment of the disclosed glucosamine compositions.

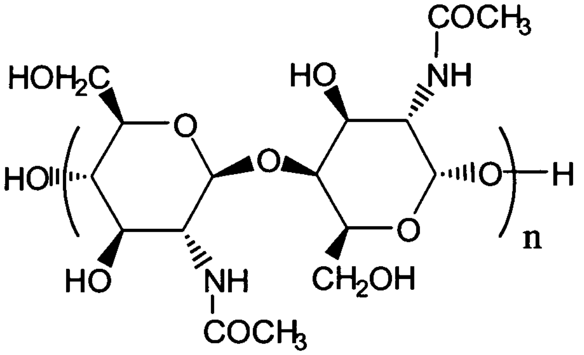
FIG. 6 is a chromatogram of an embodiment of the disclosed glucosamine compositions.

FIG. 7 is an FTIR spectra showing comparison of certain of the presently disclosed glucosamine compositions to glucosamine materials derived from shellfish.

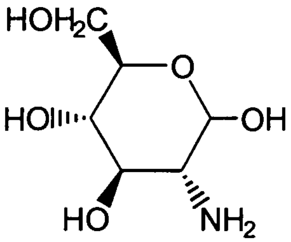
FIG. 8 is an HPLC chromatogram that compares water-soluble components of an embodiment of the disclosed composition to glucosamine derived from fungal biomass indicating that no levulinic acid or dextrose was detected in the shellfish-derived glucosamine.

Detailed Description

Disclosed are glucosamine compositions and glucosamine composition products, such as food supplements, suitable for human or animal consumption. The glucosamine compositions are derived from chitin present in various types of fungal biomass. Chitin is a natural polysaccharide, with the structure of an unbranched polymer of 2-acetoamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine). The formula for chitin can be represented by the general repeating structure:



Chitin is typically an amorphous solid that is largely insoluble in water, dilute acids, and alkali. Although chitin has various commercial applications, commercial utility can be found by transforming the polymeric structure into individual components of 2-amino-2-deoxy-D-glucose, which is known as glucosamine. Structurally, glucosamine is modified glucose with an amine group replacing the OH group found on the carbon two (C-2) atom. The general structure of glucosamine is:



As stated above, glucosamine compositions disclosed herein include glucosamine derived from fungal biomass containing chitin and may include other components as well.

Suitable starting materials for producing the glucosamine compositions include substantially uniform microbial fungal sources, such as fungal sources derived from *Aspergillus sp., Penicillium sp., Mucor sp.* and combinations thereof. Use of a fungal biomass results in a high-quality product that produces glucosamine compositions having low levels of impurities, such as undesirable minerals. The glucosamine compositions normally have relatively low ash content and thus, no or at most trace levels of heavy metals. In addition, low ash content provides relatively clear solutions made from the glucosamine compositions.

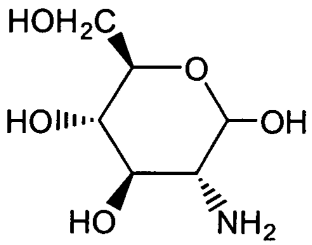
In addition, because the glucosamine compositions are products of fungal biomass, the glucosamine compositions disclosed herein are not subject to inclusion of the protein allergens found in glucosamine produced from shellfish.

A. Glucosamine Compositions

The glucosamine compositions may be derived from relatively uniform fungal biomass sources, so that the glucosamine compositions are generally uniform. "Uniform fungal biomass" refers to fungal biomass comprising substantially the same species grown on substantially the same media, grown in a relatively controlled environment or other such conditions that lead to substantial uniformity in the biochemical make-up of the biomass. Depending upon the methodology used to purify the glucosamine compositions such as desired glucosamine salt compositions, the resulting glucosamine containing compositions can be produced with varying amounts of glucosamine, including compositions that exceed 95 percent glucosamine, 98 percent glucosamine, and even 99.8 percent glucosamine. The glucosamine compositions can contain additional ingredients, such as salts, melanoidins and acids, e.g., levulinic acid (as discussed below). Certain of the glucosamine compositions include 0.01 to 10% glucose, 0.01 to 5% glucose, or 0.01 to 2% glucose.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, percentages, reaction conditions, and so forth used in the specification and claims are to be understood as being modified by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth are approximations that may depend upon the desired properties sought.

The glucosamine in the disclosed compositions has the general formula represented below:



This general formula varies in different embodiments of the glucosamine compositions depending upon the presence of various salts of the glucosamine, including citrate, acetate, phosphate, sulfate, chloride, lactate, gluconate, etc. Also, the glucosamine in the glucosamine compositions can be substituted or modified without diverging from the scope of the invention. Thus, as used herein, the term glucosamine refers to the various forms of glucosamine, including salt complexes and substituted glucosamine. Likewise, the term glucosamine composition refers to compositions including glucosamine in such various forms.

Embodiments of the glucosamine compositions include particular components in addition to glucosamine, such as glucose, unreacted chitin, and glucan conversion materials, such as melanoidins and levulinic acid.

Melanoidins are relatively complex, high molecular weight, irregular polymers and are present in particular embodiments of the glucosamine compositions. For example, particular embodiments of the disclosed glucosamine compositions include from 0.001 to 15 wt. % melanoidins, or from 0.001 to 1.0 wt. % melanoidins or from 0.01 to 0.1 wt. % melanoidins. Without being tied to any particular theory, melanoidins are likely formed by the conversion of glucans to dextrose to hydroxymethylurfural (HMF) to produce the melanoidins. (The reaction may produce other glucan-derived products and amines from proteins in a biomass source as well as lipids in such a source.) Such a chemical process is known as the Maillard Reaction.

Levulinic acid (also known as acetyl-propionic acid) is present in particular embodiments of the disclosed glucosamine compositions. Without being tied to any particular theory, levulinic acid is likely formed when glucans in the fungal biomass are converted to dextrose, which is converted to HMF to finally form formic and levulinic acids. Levulinic acid is a non-hazardous component that is a valuable acidulant used in such products as carbonated and fruit juice beverages, jams, and jellies. Thus, addition of embodiments of the glucosamine compositions to such products provides an acidulant benefit as well as the benefits provided by the glucosamine in the composition. Particular embodiments of the glucosamine compositions include from 0.0001 to 1 wt. % levulinic acid, or from 0.001 to 0.7 wt. % levulinic acid or from 0.01 to 0.4 wt. % levulinic acid.

Because the melanoidins and levulinic acid are formed when producing the glucosamine compositions according to the disclosed methods, no additional steps must be taken to include such components in the compositions. Melanoidins and levulinic acid were not expected in glucosamine compositions derived from shellfish, and analysis of six lots of glucosamine derived from shellfish (obtained from five different suppliers) did not contain any detectable amounts of melanoidins or levulinic acid.

As discussed, complex carbohydrates in fungal biomass, such as glucans, are converted to melanoidins in the reducing environment of the process. These complex carbohydrates are not present in the shellfish carapaces used in other processes, and so the melanoidins do not form. Comparison of FTIR spectra (FIG. 7) of water-insoluble materials in certain embodiments of the disclosed glucosamine compositions to those found in a typical shellfish-derived glucosamine shows that melanoidins are not present in shellfish derived glucosamine compositions. The FTIR spectrum of the insoluble material from the disclosed glucosamine composition has several broad bands with no fine structure, typical of polymeric materials. The bands between 2800 and 3000 wave numbers in the spectrum of the present compositions are typical of amide groups in melanoidins. The insoluble material from the shellfish derived glucosamine product has no such indications of the presence of melanoidins in the FTIR spectra.

Because melanoidins are irregular polymers with reduced carbon, some degree of conjugation exists between the pi bonds. This conjugation results in the typical tan to brown color of melanoidins. Such coloration was clearly present in embodiments of the presently disclosed glucosamine compositions but was absent in the shellfish-derived glucosamine samples again indicating that shellfish derived glucosamine compositions do not include melanoidins.

Melanoidins are reported to possess antioxidant and/or free radical scavenging character. See, e.g., Gow-Chin Yen, et al., *Antioxidant Activity and Scavenging Effects on Active Oxygen of Xylose-Lysine Maillard Reaction Products*, J. Sci. Food Agric., 67, 415-420 (1995); K. Eichner, *Antioxidant Effect of Maillard Reaction Intermediates*, Prog. Fd. Nutr. Sci., 5, 441-451 (1981); Fumitaka Hayase, et al., *Scavenging of Active Oxygens by Melanoidins,* Agric. Biol. Chem, 53(12), 3383-3385 (1989); Dejian Huang, et al., *High-Throughput Assay of Oxygen Radical Absorbance Capacity (ORAC) Using a Multichannel Handling System Coupled with a Microplate Fluorescence Reader in 96-Well Format,* J. Agric. Food Chem., 50, No. 16, 4437-4444 (2002), each of which is incorporated herein by reference. Certain embodiments of the glucosamine compositions disclosed have lipophilic oxygen radical absorbance capacity values (lipo-ORAC values) of from 30 μmole TE/g (TROLOX equivalent per gram) to 150 μmole TE/g or lipo-ORAC values of from 35 μmole TE/g to 100 μmole TE/g or from 35 μmole TE/g to 50 μmole TE/g. TROLOX is also known as 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid.

The lipo-ORAC values may be determined, e.g., by use of an ORAC assay using fluorescein (FL) as a fluorescent probe as discussed in Dejian Huang, et al., *Development and Validation of Oxygen Radical Absorbance Capacity Assay for Lipophilic Antioxidants Using Randomly Methylated β-Cyclodextrin as the Solubility Enhancer,* J. Agric. Food Chem., 50, No. 7 (2002), which is incorporated herein by reference. Randomly methylated β-cyclodextrin (RMCD) is used as a water solubility enhancer for lipophilic antioxidants. Seven percent RMCD (w/v) in a 50% acetone-water mixture is used to solubilize the lipophilic antioxidants in 75 mM phosphate buffer (pH 7.4). When using TROLOX as the standard (1.0), α-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol, α-tocopherol acetate, tocotrienols, 2,6-di-*tert*-butyl-4-methylphenol, and γ-oryzanol have ORAC values of 0.5 +/- 0.02, 0.74 +/- 0.03, 1.36 +/- 0.14, 0.00, 0.91 +/- 0.04, 0.16 +/- 0.01, and 3.00 +/- 0.26, respectively, when using this method.

Levulinic acid and dextrose, present in certain embodiments of the disclosed glucosamine compositions are not expected to be present in glucosamine derived from shellfish. High performance liquid chromatography demonstrates the differences between embodiments of the glucosamine composition disclosed herein and shellfish-derived glucosamine compositions. Neither levulinic acid nor dextrose was detected in any shellfish-derived glucosamine products.

Specifically, samples of the present compositions and shellfish-derived glucosamine compositions were dissolved in 0.01 N sulfuric acid at a concentration of 4% w/v. Diluted samples were filtered through 0.2 mm nylon filters into HPLC vials. Chromatograms were collected using a Metacarb H Plus column (Varian, Inc., Torrence, CA) using 0.01 N sulfuric acid as the eluent at 0.4 mL/min. Peaks were identified by retention time against known standards. As is apparent in FIG. 8, levulinic acid and dextrose were present only in the presently disclosed glucosamine compositions and not in the shellfish derived compositions.

With reference to Table 1, embodiments of the glucosamine compositions comprise glucosamine derived from fungal biomass and may also comprise one or more of the listed components in Table 1, those shown in Table 2 and other components as discussed herein. Concentrations of each component may be within the ranges shown or may be varied by altering any of a variety of production parameters.

Table 1

Glucosamine Composition Components	Representative Embodiment Percent by Weight	Representative Embodiment Percent by Weight	Representative Embodiment Percent by Weight
Glucosamine	85-99.8	95-99.8	98-99.8
Melanoidins	0.001-15	0.001-1.0	0.01-0.1
Levulinic Acid	0.0001-1	0.001-0.7	0.01-0.4
Dextrose	0.001-10	0.001-5	0.001-2
Citric Acid	0.001-10	0.01-1.0	0.025-0.5

With reference to Table 2, two specific embodiments of the glucosamine compositions are set forth. The methods utilized to determine the components present and concentrations of the same are set forth below.

Table 2

Composition Component	*Embodiment 1 (GP-11)	*Embodiment 2 (GP-17C)
Ash Content	0.03%	0.02%
Si	140 ppm	150 ppm
Na	10-100 ppm	10-100 ppm
K	10-100 ppm	10-100 ppm
Ca	10-100 ppm	10-100 ppm
HCL	0.16%	0.19%
Citric Acid	0.045%	0.074%
Levulinic Acid	0.39%	0.3%
Melanoidins	0.04-0.07%	0.02-0.03%
Water-insoluble matter soluble in gastric juice at ~40°	0.05%	0.02%

*Percentages listed are percents by weight

Certain embodiments of the glucosamine compositions have relatively low ash content. The ash content may be less than 5 percent, less than 2 percent, or less than 1 percent. There are little if any heavy metal components in the glucosamine compositions; the heavy metal component concentrations in the disclosed glucosamine compositions are well below 100 parts per million, more typically below 50 parts per million, even more typically below 20 parts per million. In certain embodiments the heavy metal components are present in less than 10 parts per million.

The glucosamine component of the glucosamine compositions can have a positive specific rotation, such as a positive 69 to 74 degree specific rotation for the glucosamine hydrochloride salt. The glucosamine compositions are usually relatively white when in purified dry form, but colorless when dissolved in an aqueous solution. In one example, a 20 percent by weight solution of the glucosamine has an American Public Health Association (APHA) color of less than 50.

The glucosamine compositions may also be combined with further components to form a food supplement for human and/or animal ingestion. For example, the glucosamine compositions may be further combined with excipient, common pharmaceutical binders (e.g., sucrose, glucose, ethyl cellulose, methyl cellulose, polyvinyl pyrrolidone, polyethylene glycol, lactose, dicalcium phosphate, crosprovidone, croscarmellose, and the like), common organic acids (e.g., citric acid, malic acid, tartaric acid, lactic acid, and the like), and/or carbohydrates (e.g., starch, glucose, sucrose, and the like). Such glucosamine compositions may also be combined with sugars, artificial sweeteners, natural and artificial colors, natural and artificial flavorings, acidulants, thickeners, and the like, to form a variety of food supplements. Such glucosamine composition food supplements are typically made into food supplement beverages, bars, concentrates, dry or concentrated drink mixes, powders, chews, confections, gums, yogurts, patches, lozenges, and the like.

B. Microbial Fungal Biomass Starting Materials

Suitable starting materials for producing the disclosed glucosamine compositions include microbial biomass sources, typically fungal biomass, such as filamentous fungi having greater than 10 percent chitin by total dry cell weight, such as fungal sources derived from *Aspergillus sp., Penicillium sp., Mucor sp.* Suitable fungal biomasses include *Aspergillus niger, Aspergillus terreus, Aspergillus oryzae, Mucor rouxii, Penicillium chrysogenum, Penicillium notatum, Saccharomyces cerevisiae; Saccharomyces uvarum*; and in particular *Candida guillermondi, Aspergillus niger,* and *Aspergillus terreus.* The biomass may be recovered from a commercial fermentation reaction, such as the commercial production of organic acids, including citric acid. Also, biomass suitable for production of glucosamine can be generated specifically for this process and not as a byproduct of other processes. As used herein, the term microbial does not include phyto-plankton and crustaceans or mollusks.

Biomasses having chitin levels in excess of 5 percent of the dry biomass weight are suitable for practicing the methods disclosed. Such biomass usually has between 5 and 25 percent chitin, and can have from 10 to 20 percent chitin, based upon dry weight of the biomass. Also, in order to prepare food or supplemental grade glucosamine compositions it is sometimes desirable that the microbial biomass be grown in a substantially controlled manner having relatively uniform temperature and/or nutrient levels during the growth of the biomass. Nutrient levels can be controlled by any suitable manner, for example as disclosed in U.S. Patent Nos. 2,739,923, 2,353,771, and 2,674,561, which are incorporated herein by reference.

C. Methods for Producing Fungal Biomass Glucosamine Compositions

Also disclosed are methods for producing glucosamine compositions from fungal biomass sources, including producing such compositions by acid hydrolysis of fungal biomass. Acid hydrolysis breaks ether linkages in the biomass and deacetylates chitin molecules to generate free glucosamine. Acid hydrolysis can break the chitin into glucosamine, but leaves the glucosamine molecule substantially intact. Depending upon the acid hydrolysis parameters, acid hydrolysis conditions break down other components (such as glucans, proteins, and lipids) that exist in the fungal biomass.

In one specific of the disclosed method for producing glucosamine compositions from fungal biomass, acid hydrolysis is performed by treating fungal biomass for a relatively long period of time, for example greater than 4 hours, in a relatively aggressive acid solution.

With reference to FIG. 2, chitin-containing fungal biomass (a) may first be reacted in a relatively aggressive acidic solution (c). Relatively strong (aggressive) acids may be used to hydrolyze the fungal biomass, including acids of concentrations less than 50 percent. Acids of concentrations of from 5 to 25 percent are also suitable. Suitable strong acids include hydrochloric, sulfuric, phosphoric, and citric acid at appropriate concentrations.

In particular embodiments of the disclosed methods particular glucosamine compositions are formed by an aggressive acid treatment, reacting from 5 to 20 percent acid with from 2 to 50 percent pretreated biomass (based upon dry weight, although the biomass is typically processed with water present) and from 35 to 93 percent water. In certain implementations the reaction mixture comprises from 8 to 12 percent hydrochloric acid, from 4 to 8 percent biomass (based upon dry weight), and from 80 to 90 percent water. In yet another embodiment, the acid solution is from 17 to 20 percent hydrochloric acid solution.

The aggressive acid treatment mixture containing the biomass, acid, and water is heated and maintained at a relatively elevated temperature. The mixture is usually heated to a temperature at or near its boiling point (typically 90 ° C to 106 ° C) and maintained under reflux conditions for 5 hours or greater, more typically greater than 8 hours, and usually less than 16 hours. The reaction may continue long enough to have a complete breakdown of the chitin, but not so long as to be inefficient or to excessively decompose the glucosamine compositions.

Although reaction in the relatively aggressive acid solution produces a glucosamine composition, subsequent purification steps may be taken. A first purification step may include a separation step, such as filtration, to remove particulate impurities, resulting in a substantially clear solution of the glucosamine composition, (d) in FIG. 2. The solution contains an embodiment of glucosamine composition as well as small quantities of glucose and other components of the composition. The glucosamine composition can be concentrated and some of the acid recovered can be recycled and reused.

The glucosamine composition may be crystallized, (e) in FIG. 2. For example, the glucosamine composition may be crystallized by adding ethanol to the concentrated solution or by continuing evaporation to the glucosamine composition solubility limit.

The glucosamine composition can be recovered by a separation process, such as filtration or centrifugation, followed by drying. The dried glucosamine composition is optionally further treated to remove undesirable residual sugars. One method of removing such sugars is by dissolving the glucosamine composition in water and adding ethanol to again precipitate the glucosamine composition while undesirable sugars remain in solution. Alternatively, the solution can be treated by electro dialysis, chromatography, membrane filtration, or other suitable procedures to further increase the concentration of glucosamine in the glucosamine composition. The glucosamine composition may optionally be decolorized and/or deodorized by, for example, treating the composition with ethanol, carbon, or other suitable material or method.

Such an aggressive acid hydrolysis method typically has a yield of glucosamine composition of greater than 50 percent of the total chitin content of the fungal biomass starting material.

In an alternative embodiment of the method set forth above, the biomass can initially be treated to remove some impurities and/or to improve glucosamine composition production. These treatments can include, for example, heating the biomass, adding digestive enzymes, mixing with an acid or base, mechanical agitation, or dewatering by compression. One optional treatment for removing proteins, lipids, and residual citric acid involves pretreating the biomass in the presence of a base, such as sodium hydroxide ((b) in FIG. 2).

In certain embodiments a concentration of less than 10 percent sodium hydroxide is added to the fungal biomass. The basic solution is heated to a relatively elevated temperature for a period of time sufficient to remove a desirable amount of the non-chitin containing material, such as proteins and lipids. This period of time may be less than two hours. One specific example of this pretreatment method involves heating the fungal biomass to from 100° to 125 °C in a 1 to 8 percent solution of sodium hydroxide for 20 to 60 minutes. Alternatively, the sodium hydroxide concentration may be 1 to 4 percent. Embodiments wherein the biomass is treated with a basic solution, protein and glucans are hydrolyzed in the biomass. These byproducts may optionally be removed by, for example, filtration. The removal of such proteins and other waste products may be followed by treatment to remove soluble proteins, amino acids, and other impurities.

An alternative to treating the biomass with a basic solution could include, for example, treating the fungal biomass in solution with protease enzymes or other suitable enzymes to remove undesirable components such as proteins and lipids. Yet another alternative embodiment comprises mechanically treating the fungal biomass to physically break down the cell walls so that undesirable proteins and lipids within the cells can be removed prior to extracting the chitin from the cell walls themselves. In yet another alternative embodiment, alcohols are used to remove undesirable components from the fungal biomass prior to acid hydrolysis.

In another embodiment of the method for producing glucosamine compositions from fungal biomass, the biomass material may undergo a mild acid pre-treatment followed by an aggressive acid treatment.

More specifically, with reference to FIG. 3 chitin-containing biomass (a) may first undergo a mild acid pre-treatment (d). The acid hydrolysis conditions (parameters comprising time, temperature, and acid concentration) used are "mild" in comparison to the subsequent aggressive acid treatment (f). The acid hydrolysis that occurs under the relatively mild conditions allows removal of undesirable constituents from the biomass prior to the aggressive acid treatment (f). A mild acid treatment therefore may be used to improve any one of several aspects of producing the glucosamine composition from fungal biomass. A mild acid can be used to break down the cell walls of the fungal biomass such that extraneous biomass constituents, such as proteins, lipids and undesirable polysaccharides can be removed prior to hydrolyzing the chitin. The acid concentration during mild acid treatment may be from 0.05 to 20% or 0.1 to 12%, or from 0.5 to 5% w/w acid, such as HCl. As with the aggressive acid treatment acid percentage ranges vary depending upon the type of acid used. For example, citric acid will require higher percentage ranges while HC1 is suitable at the ranges set forth above. Higher concentrations of strong acid solutions or the use of different acids or mixed acids may be used to break down the cell walls more quickly, yet reaction conditions must be adapted to control the undesirable, premature conversion of the chitin to glucosamine. Likewise lower concentrations of strong acids, weak acids or mixed acids may be used (especially at relatively higher temperatures, for longer time periods, or at higher concentrations) such that the cell walls are sufficiently broken down to afford removal of a substantial or desirable portion of the extraneous biomass constituents, e.g., lipids, proteins and undesirable polysaccharides.

A mild acid treatment (d) may be performed by reacting the following components: from 0.05 to 20 percent acid, and from 1 to 50 percent biomass (based upon dry weight). In certain implementations the mild acid reaction mixture comprises from 0.1 to 12 percent hydrochloric acid, and from 3 to 25 percent biomass (based upon dry weight). In yet another embodiment the solution amounts comprise from 0.5 to 5 percent hydrochloric acid and from 5 to 15 percent biomass (based upon dry weight).

The mild acid treatment may be carried out at a temperature of 60°C to reflux temperature or from 70°C to 105°C, or at a temperature of 80°C to 100°C. Higher temperatures may be used as long as it is not so high as to convert a significant amount of the chitin to glucosamine. Likewise, lower temperatures (such as 60°C-90°C) may be used (especially with relatively concentrated acids) as long as the cell walls are sufficiently broken down to release the waste products, e.g., lipids, proteins, and undesirable polysaccharides, without converting a significant amount of chitin to glucosamine. As used herein “a significant amount of chitin to glucosamine” means less than an amount that would provide a low yield of glucosamine in the final glucosamine composition, less than 10% of the chitin, or less than 5% of the chitin, or less than 2% of the chitin.

Prior to or following the mild acid treatment, the fungal biomass (a) (or the solids (e) retained after the mild acid treatment (d) removal of the undesirable products) may optionally be treated with a mildly basic solution (b) as described above and as referenced in FIG. 3. Although method steps are shown and described in specific orders, it is to be understood that the order of these steps may be varied without departing from the disclosed methods.

The solids (e) retained after the mild acid treatment (and optionally the mild base treatment (b)) are then treated with an aggressive acid (f) as discussed in the embodiment above. In this embodiment, however, a large portion of the impurities, primarily glucans, have already been removed from the solution (between steps (d) and (e)). Accordingly, the aggressive acid treatment (f) to convert chitin in the remaining solids from the fungal biomass to a glucosamine composition requires significantly less acid. For example, with an aggressive acid treatment under conditions such as 17% HCl and 10% dry biomass solids for 9 hours at 100°C, the hydrochloric acid needed in the aggressive acid step could be reduced by from 20 to 60%.

When a mild acid treatment and waste product removal process is performed prior to an aggressive acid treatment, because less acid need be used, the amount of final resulting waste solution (between steps (h) and (i)) is a significantly smaller volume as compared to the method omitting the mild acid treatment. The acid needed to treat the biomass is typically extremely expensive; a smaller volume of acid is a significant cost savings, especially when producing the product on a commercial scale. The smaller volume of acidic solution also allows for smaller separation apparatus to separate the glucosamine composition from the acidic solution. Because apparatus needed to separate such a concentrated acid solution must be formed of special (and expensive) materials resistant to the corrosive activities of concentrated acids, smaller separation apparatus saves a significant amount in costs of manufacturing glucosamine compositions from fungal biomass, especially on a commercial scale. When a mild acid treatment precedes the aggressive acid treatment the smaller volume of acidic solution results in less waste solution to be treated once the glucosamine composition is removed therefrom.

Glucosamine compositions are formed during the aggressive acid treatment following a mild acid treatment in the same manner as compositions formed with aggressive acid treatment alone.

When the chitin in the remaining solid (e) is treated with the aggressive acid (f), glucans not removed in the preceding separation process are converted to beneficial glucosamine composition components, such as melanoidins and levulinic acid. To alter the concentrations of such components of the glucosamine composition, one may allow more of the glucans to remain in the remaining solid (e).

Process steps following the aggressive acid treatment (f) are substantially similar to those discussed above.

In yet another embodiment of the methods for producing glucosamine compositions from fungal biomass, increased temperatures and/or pressures are utilized with an aggressive acid treatment. This allows the reaction to occur using less acid or in a shorter time period than the above-mentioned aggressive acid treatment. Temperature ranges for this the increased temperature, aggressive acid treatment are from 90°C to 160°C, for example, from 105°C to 160°C. The pressure may be allowed to build as a function of reactions taking place in a sealed vessel.

More specifically, fungal biomass is treated at the aggressive acid treatment phase with an acid, such as from 4 to 20% acid or from 6 to 13%. The lower concentrations of acid still convert the chitin in solution to glucosamine because the reaction conditions are changed to increase the temperature and/or the pressure parameters. Specifically, the acid/biomass solution is placed in a sealed vessel such that the reaction may take place at pressures of slightly over atmospheric to 10 atmospheres, or slightly over atmospheric to 4 atmospheres, such as at 2 atmospheres. The increased pressures may be due to the reaction taking place at an increased temperature in a sealed vessel or the reaction may take place in a vessel in which the pressure is otherwise made to increase.

The temperature, if elevated, is preferably from 90°C to 160°C, or 100°C to 140°C, such as 110°C to 130°C. The reaction may take place at such elevated temperatures at the pressures set forth above or outside a closed vessel at atmospheric pressure. If the temperature of the reaction takes place at from 90°C to 160°C in a closed vessel, the pressures will generally be at atmospheric pressure to 5 atmospheres (65 psig). Good results are obtained with, e.g., a reaction temperature of 120°C and a pressure of 1 atmosphere (or 15 psig).

Other methods of increasing the temperature are available and included in the methods proposed, for example, increasing the boiling point by adding salts.

The remainder of the increased temperature and/or pressure methods for producing glucosamine compositions from fungal biomass follows those steps outlined in the above-described methods (such as shown in FIGS. 2 or 3). Specific examples of the increased temperature and/or pressure methods for producing glucosamine compositions are set forth below.

D. Examples

The invention will be further explained by the following non-limiting illustrative examples. Unless otherwise indicated, all amounts are expressed in parts by weight.

Example 1

Citric biomass was pretreated with a 4 percent aqueous sodium hydroxide (NaOH) solution in an autoclave at 120 °C for 1 hour. This step removed excess proteins and other undesirable materials. The biomass was then thoroughly washed with de-ionized water until its pH was approximately 7.0. This washed material was mixed with concentrated hydrochloric acid (HCl) and water to form a mixture of 10 to 15 percent HCl and 5 to 6 percent biomass, based upon dry weight of the biomass. This mixture was heated at reflux. Samples were taken from time to time, and the reaction analyzed with a high-pressure liquid chromatograph available from Dionex HPLC under the trade designation "DX-500".

The results are provided in FIG. 4, which shows a chart indicating glucosamine production, and shows that the glucosamine was increasingly produced as the reaction ran through 8 hours, but that the amount of glucose diminished after 4 hours. After 8 hours the glucosamine produced in the yield of 14 percent. A chromatogram of the product is shown in FIG. 5.

Following reaction, the mixture was filtered, and the filtrate evaporated using a rotating evaporator manufactured by RotaVap to increase the glucosamine concentration of the solution. The final volume was reduced to 10 to 20 ml. To this solution was added 20 ml of ethanol and the solution swirled to promote precipitation of glucosamine and enhance yield. These glucosamine precipitates were obtained by filtration and were further washed with alcohol until the color became white. FIG. 6 shows a chromatogram of the product, indicating greater than 97 percent glucosamine in the glucosamine composition.

Example 2

Example 1 was repeated, but the pretreated biomass was maintained under reflux conditions for 13 hours. The resulting glucosamine composition contained greater than 98 percent glucosamine.

The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood from this description or examples. The invention is not limited to the exact details shown and described, for variations will be included within the invention defined by the claims.

Example 3

Filtered biomass (3900g) from a citric acid production process was combined with 100 mL concentrated hydrochloric acid and 4.5 L water. The resulting solution (0.5% HCl, 7.8% biomass solids) was maintained at 90-100°C for 2 hours. The reaction mixture (71.9g) was filtered and washed with 5 portions of water at 60-70°C for a total of 400 mL wash. The washed biomass solids weighed 31.5 g and were found to contain 12.5% solids upon drying. The washed biomass solids therefore contained 3.9 g solids out of 71.9 g, or 5.4% solids after mild acid treatment as described above. When compared to the initial 7.8% solids prior to the mild acid treatment a 31% reduction in biomass solids was calculated.

To estimate the amount of the desirable component of the filtered biomass (chitin) sacrificed during the mild acid treatment an aggressive acid treatment was conducted using both pretreated and non-pretreated biomass to produce glucosamine hydrochloride in the following manner:

Dried (pretreated or non-pretreated) biomass (0.40 g) was combined with 3.60 g of 22.5% hydrochloric acid in a small test tube. The resulting solutions (20%HCl, 10.0%

biomass solids) were held at 105°C for 2.5 hours in a heat block. Dionex HPLC analysis of the two acid hydrolyzed samples allowed the percent glucosamine hydrochloride by weight to be determined and compared. Specifically, the amount of free glucosamine was determined using high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The system consisted of an EG40 eluent generator, GP50 gradient pump, AS40 autosampler, LC25 column oven, and ED40 electrochemical detector, all produced by Dionex Corporation, Sunnyvale, California, U.S.A. The method was adapted from Dionex Corporation Technical Note 40, incorporated herein by reference. A Dionex CarboPac PA-20 column was used rather than a PA-10 column. The eluent was 8 mM KOH at 0.5 mL/min. The column and detector were maintained at 30°C. The injection volume was 10 µL. The standard was glucosamine hydrochloride at 10.8 mg/L. Samples were diluted with deionized water, ASTM Type II, and filtered through 0.2 µm vial filters in an autosampler. Multiple standards were analyzed before and after each sample set.

The non-pretreated biomass sample contained 2.1% glucosamine hydrochloride. The maximum theoretical amount of glucosamine hydrochloride attainable from the pretreated biomass is 3.0% (assumes all 31% reduction in biomass solids is non-chitin). The pretreated biomass sample was measured at 2.7% glucosamine hydrochloride by weight. Thus, mild acid pretreatment resulted in a 29% chitin-enrichment of the biomass solids, yet reduced the yield of glucosamine hydrochloride from the original biomass by 10%.

Example 4

Filtered biomass (3900 g) from a citric acid production process was combined with 100 mL concentrated hydrochloric acid and 4.5 L water. The resulting solution (0.5% HCl, 7.8% biomass solids) was held at 90-100°C for 20 hours. The reaction mixture (95.2 g) was filtered and washed with 5 portions of water at 54-70°C for a total of 320 mL wash. The washed biomass solids weighed 26.9 g and were found to contain 16.0% solids upon drying. The washed biomass solids therefore contained 4.3 g solids out of 95.2 g, or 4.5% solids after a mild acid treatment as described above. When compared to the initial 7.8% solids prior to the mild acid treatment one can calculate a 42% reduction in biomass solids was obtained.

To estimate the amount of the desirable component of the filtered biomass (chitin) sacrificed during the mild acid treatment, an aggressive acid treatment was conducted using both pretreated and non-pretreated biomass to produce glucosamine hydrochloride in the following manner:

Dried (pretreated or non-pretreated) biomass (0.40 g) was combined with 3.60 g of 22.5% hydrochloric acid in a small test tube. The resulting solutions (20% HCl, 10.0% biomass solids) were held at 105°C for 2.5 hours in a heat block. Dionex HPLC analysis (performed as described in Example 4) of the two acid hydrolyzed samples allowed the percent glucosamine hydrochloride by weight to be determined and compared. The non-pretreated biomass sample contained 2.1% glucosamine hydrochloride. The maximum theoretical amount of glucosamine hydrochloride attainable from the pretreated biomass is 3.6% (assuming all 42% reduction in biomass solids is non-chitin). The pretreated biomass sample was measured at 3.0% glucosamine hydrochloride by weight. Thus, mild acid pretreatment resulted in a 43% chitin-enrichment of the biomass solids yet reduced the yield of glucosamine hydrochloride from the original biomass by 17%.

Example 5

Filtered biomass (2000 g) from a citric acid production process was combined with 3000 g of a 7.5% hydrochloric acid solution. The resulting solution (4.5% HCl, 6.0% biomass solids) was held at 90-100°C for 2 hours. A portion (40.7 g) of the reaction mixture was transferred to a 50 mL centrifuge tube. The sample was centrifuged and the liquor was decanted. The remaining solids were subsequently washed five times with 25-30 mL portions of NaOH solution (pH 13.1) then washed four times with 25 mL portions of HCl solution (pH 1.3). A final adjustment of the pH to near neutral afforded the isolation of washed biomass solids by decantation. The biomass solids weighed 5.9 g and were found to contain 14.2% solids upon drying. The washed biomass solids therefore contained 0.84 g solids out of 40.7 g, or 2.1% solids after mild acid treatment as described above. When compared to the initial 6.0% solids prior to the mild acid treatment a 65% reduction in biomass solids was calculated.

To estimate the amount of the desirable component of the filtered biomass (chitin) sacrificed during the mild acid treatment an aggressive acid treatment was conducted using both pretreated and non-pretreated biomass to produce glucosamine hydrochloride in the following manner:

Dried (pretreated or non-pretreated) biomass (0.10 g) was combined with 1.90 g of 20.3% hydrochloric acid in a small test tube. The resulting solutions (19.3% HCl, 5.0% biomass solids) were held at 105°C for 4 hours in a heat block. Dionex HPLC analysis (performed as described above) of the two acid hydrolyzed samples allowed the percent glucosamine hydrochloride by weight to be determined and compared. The non-pretreated biomass sample contained 1.0% glucosamine hydrochloride. The maximum theoretical amount of glucosamine hydrochloride attainable from the pretreated biomass is 2.9% (assuming all 65% reduction in biomass solids is non-chitin). The pretreated biomass sample was measured at 2.1% glucosamine hydrochloride by weight. Thus, mild acid pretreatment resulted in a 110% chitin-enrichment of the biomass solids, yet reduced the yield of glucosamine hydrochloride from the original biomass by 28%.

Example 6

Filtered biomass (3000 g) from a citric acid production process was combined with 3000 g of 8.7% sodium hydroxide solution. The resulting solution (4.4% NaOH, 8.1% biomass solids) was held at 90-100°C for 45 minutes. The reaction mixture was filtered and washed with water at 40-50°C until the percent NaOH remaining in the washed biomass solids was less than 0.06%. The washed biomass solids weighed 1479 g and were found to contain 22.9% solids upon drying. The washed biomass solids therefore contained 339 g solids out of 6000 g or 5.7% solids after mild base treatment as described above. When compared to the initial 8.1% solids prior to the mild base treatment a 30% reduction in biomass solids was calculated.

The washed biomass solids obtained from mild base treatment were subsequently subjected to a mild acid treatment. The washed biomass solids (1310 g) was combined with 3665 g of 5.5% hydrochloric acid solution and 25 g of glacial acetic acid. The resulting solution (4.0% HCl, 0.5% acetic acid, 6.0% biomass solids) was held at 90-100°C for 3.5 hours. At this time a portion, 944 g, of the reaction mixture was filtered and washed with 1409 g water in two portions. The washed biomass solids weighed 298 g and were found to contain 12.5% solids upon drying. The washed biomass solids therefore contained 37.3 g solids out of 944 g, or 4.0% solids after mild acid treatment. When compared to the initial 6.0% solids of the mild acid treatment a 33% reduction in biomass solids was calculated. An overall reduction of 53% in biomass solids resulted from the combined effect of mild base treatment followed by mild acid treatment.

To estimate the amount of the desirable component of the filtered biomass (chitin) sacrificed during the mild base and mild acid treatments, an aggressive acid treatment was conducted using both pretreated and non-pretreated biomass to produce glucosamine hydrochloride in the following manner:

Dried (pretreated or non-pretreated) biomass (0.10 g) was combined with 1.90 g of 22.8% hydrochloric acid in a small test tube. The resulting solutions (21.6% HCl, 5.1% biomass solids) were held at 105°C for 4 hours in a heat block. Dionex HPLC analysis (performed as described above) of the three acid hydrolyzed samples allowed the percent glucosamine hydrochloride by weight to be determined and compared. The non-pretreated biomass sample contained 0.92% glucosamine hydrochloride. The maximum theoretical amount of glucosamine hydrochloride attainable from the mild base pretreated biomass is 1.3% (assuming all 30% reduction in biomass solids is non-chitin). The mild base pretreated biomass sample was measured at 1.3% glucosamine hydrochloride by weight. Thus, mild base pretreatment resulted in a 41% chitin-enrichment of the biomass solids without a reduction in the yield of glucosamine hydrochloride from the original biomass. The maximum theoretical amount of glucosamine hydrochloride attainable from the mild acid pretreated biomass is 2.0% (assumes the overall 54% reduction in biomass solids is non-chitin). The mild acid pretreated biomass sample was measured at 1.5% glucosamine hydrochloride by weight. Thus, mild acid pretreatment following the mild base pretreatment resulted in a 63% chitin-enrichment of the original biomass solids, yet reduced the yield of glucosamine hydrochloride from the original biomass by 25%.

Example 7

A biomass sample from a citric acid fermentation process was combined with HCL to form a slurry of 13% HCl and 10.5% biomass solids. The slurry was placed in a sealed reactor and brought to 113°C for 10 hours. Samples of the resulting composition were taken at one hour intervals and were analyzed for glucosamine. These results were then converted to a yield based on the theoretical amount of chitin in the biomass.

This procedure was repeated using slurries of 11% and 9% HCl, with biomass solids of 12%. The results are shown in the following table.

% wt/wt HCl	**Average Temperature, °C**	**Average Pressure, psig**	**Time in hours**	**% yield based on original biomass theoretical chitin**
13	113	13	5.3	79
11	113	13	6.8	75
9	113	12	11	70

Example 8

Citric acid fermentation biomass (*A. niger)* was mixed with hydrochloric acid (JT Baker's 37 percent Reagent Grade) and placed in a sealed small scale microwave digestion bomb, available from Alltech. Prepared samples were placed in a laboratory vacuum oven with no vacuum applied. The oven was capable of maintaining a temperature of 160°C. Samples were prepared and treated under the conditions listed in Table 3 below.

Samples were diluted with nanopure water to a concentration range of within the standard range (< 10 mg/L GAP) using a Dionex HPLC system (performing the analyses as described above). Specifically, two dilutions were performed, a 1:50 dilution followed by a 1:6 dilution. The diluted samples were filtered through a 0.45 μm filter and analyzed for dextrose and glucosamine concentrations using a Dionex HPLC system.

The results are tabulated in Table 3 below. Because each trial had (at the most) four sample points, the highest glucosamine results for each trial were recorded. The sample results were not corrected for any evaporative losses in the sample during the reaction.

Table 3

Acid Conc (wt. %)	Biomass Conc (wt. %)	Time (hours)	Temperature (°C)	% yield based on original biomass theoretical chitin
2.2	3.9	4	160	18.2

2.2	3.9	4	160	10.2
2.2	3.9	2.5	160	12.6
2.2	3.9	7	140	11.6
6.2	5.2	5.5	140	17.0
6.2	10.1	5.5	140	17.9
5.7	5.8	6	160	16.3
6.2	10.0	4	140	18.4

*Percent yields based on 24% theoretical chitin in dry biomass
Glucosamine yields are not adjusted for evaporative losses. The evaporative loss is shown to provide an indication of a source of error in the bench top test.

The results of these embodiments of the glucosamine compositions as shown in Examples 8 and 9 indicate that using the disclosed increased temperature and or pressure methods for making the same indicate that significantly lower amounts or concentrations of hydrochloric acid are required to produce significant yields of the glucosamine compositions.

For all sample points selected for Table 3 the dextrose concentrations were close to zero.

Example 9

A variety of embodiments of food supplements incorporating particular embodiments of the glucosamine composition is shown in Table 4 below. The food supplements in these particular examples are in tablet, capsule, chewable, liquid, or food bar, form but could be in any suitable food supplement physical form.

Table 4

Tablet Composition Components	%
Glucosamine HCL	57
Binder	40
Dispersant	2
Flow Enhancer	0.7
Lubricant	0.3
Juice-Based Beverage Composition Components	%
Water	92.93
43 High Fructose Corn Syrup	6.0
25% Citric Acid	0.5
Fruit Punch Flavor	0.1
Glucosamine HCL	0.312

Sodium Chloride	0.05
Carboxymethyl Cellulose	0.05
10% Red 40	0.035
Monopotassium Phosphate	0.025
Potassium Benzoate	0.00021
Chew Composition Components	%
43 High Maltose Corn Syrup	23.17
42 High Fructose Corn Syrup	18.75
Sucrose	10.19
Glucosamine HCL	16.68
Evaporated Milk	7.39
Water	7.39
Coconut oil, 92°F Melting Point	6.49
Lecithin	0.14
Glycerol Monostearate	0.14
Salt	0.3
Chocolate-coating for bar	9.29
Flavor	0.1
Nutrition Bar Composition Components	%
High Fructose Corn Syrup	20
Dark Chocolate Confectionery Wafers	20
Soy Protein Isolate	15
High Maltose Corn Syrup	10
Honey	6
Whey Protein Concentrate	7
Gerkens 10/12 Russet Plus Cocoa	5
Maltodextrin	4
Water	3
Canola Oil	4
Unsweetened Chocolate	2
Glycerine	2
Fine Flake Salt	1
Glucosamine HCL	1

Summary:

A number of reactions were done to examine increasing the temperature and/or pressure of the reaction for the production of glucosamine compositions from fermentation mycelia while, in certain examples, decreasing the reaction time. Using the disclosed methods for the glucosamine composition production, it was shown that the reaction is feasible at up to at least 160°C with a hydrochloric acid concentration as low as 2% and a reaction time of two to four hours. About 50% percent less HC1 was needed to produce these results as compared to the methods where neither the reaction temperature nor the pressure was increased. The biomass concentration in the reaction is approximately up to 20 percent biomass on a dry basis. The expected yield of glucosamine from fermentation mycelia is 15 to 19 percent based on the starting dry weight of mycelia. This is in the same range or only slightly below that of the aggressive acid method and/or the mild/aggressive acid methods set forth above.

While the methods and glucosamine compositions disclosed herein may be modified, specifics thereof have been shown by way of example and are described in detail. It should be understood, however, that the specific embodiments disclosed and described are not to be interpreted as limiting the claimed invention.